

## **USE OF ETHANOL AS PLASTICIZER FOR PREPARING SUBCUTANEOUS IMPLANTS CONTAINING THERMOLABILE ACTIVE PRINCIPLES DISPERSED IN A PLGA MATRIX**

### **FIELD OF THE INVENTION**

- 5 The present invention relates to the use of ethanol as plasticizer for preparing subcutaneous implants containing thermolabile active principles dispersed in a PLGA matrix.

### **STATE OF THE ART**

- In the current state of the art the extrusion temperature of PLGA is higher than  
10 75°C. Typically the temperature during extrusion must be 40-50°C above the Tg of the polymer to be extruded.

With this type of technique it is not possible to prepare subcutaneous implants containing a thermolabile active principle dispersed in a polylactic-glycolic acid (PLGA) matrix.

- 15 To use such a technique to prepare subcutaneous implants with active principles of this type, the extrusion temperature must be lowered. In general to lower the extrusion temperature the use of a plasticizer is widespread which allows the flexibility and the workability of the polymer to be increased following the reduction of the Tg thereof. The amount of plasticizer to be added to the polymer varies as a  
20 function of the desired effect.

- An essential requirement for a plasticizer is non-volatility. Currently modern plasticizers are organic synthetic compounds; in most cases they are esters such as adipates and phthalates. These types of products are not biocompatible and therefore cannot be used for subcutaneous implants for application to man and to  
25 mammals in general.

For other types of plasticizers such as triacetin, N-methyl-2-pyrrolidone, glycerol and formaldehyde, their toxicity to man and to mammals has not been fully ascertained.

- In preparing said type of subcutaneous implants the requirement was therefore felt  
30 for a plasticizer able to reduce the extrusion temperature of PLGA which did not present the drawbacks of the state of the art and was non-toxic.

## SUMMARY OF THE INVENTION

In particular the Applicant has found that ethanol while being a volatile substance diffuses rapidly and in a homogeneous manner in ground PLGA at a temperature higher than the T<sub>g</sub> and lower than the boiling point of ethanol and therefore  
5 subcutaneous implants using ethanol as external plasticizing agent can be prepared.

The term "external plasticizer" means a plasticizing agent to be used not in the process of preparing the subcutaneous implant by extrusion, but in a phase previous to the aforesaid preparation, or rather in the preparation phase of the  
10 "plasticized" polymer which will be subsequently used in the preparation of the subcutaneous implant.

A further aspect of the present invention is therefore a plasticized PLGA containing ethanol as plasticizing agent.

This plasticized polymer is therefore prepared using a process which comprises  
15 the following stages:

- a) grinding PLGA to obtain a ground product in which the particles have dimensions less than 250  $\mu\text{m}$ ;
- b) adding ethanol to the ground product obtained in the preceding stage in concentrations between 5 and 20 parts by weight/weight of PLGA and then  
20 heating the mixture obtained to a temperature between 45 and 65°C, until a viscous and stable gel is obtained;
- c) drying the gel obtained in step (b)
- d) grinding the dried product coming from step (c) at a temperature between -20 and +5°C;
- 25 e) optionally mixing the product originating from the preceding stage with PLGA as such which has been previously ground until a ground product of dimensions less than 250  $\mu\text{m}$  is obtained, in weight ratios between 10:90 and 99:1 respectively, at a temperature between -20 and +5°C;
- f) extruding the aforesaid mixture at 75°C;
- 30 g) grinding the extruded product at a temperature between -20°C and +5°C to obtain the PLGA plasticized with ethanol according to the present invention.

A further aspect of the present invention is a subcutaneous implant comprising an

active principle dispersed in a matrix based on PLGA plasticized with ethanol according to the present invention.

#### DETAILED DESCRIPTION OF THE INVENTION

The plasticized PLGA of the present invention generally contains ethanol in concentrations between 2 and 15%, preferably between 3 and 10%, and even more preferably between 5 and 10% by weight on the weight of PLGA.

The Applicant has in fact found that by using plasticized PLGA containing ethanol at concentrations between 2% and 3% by weight, the Tg of the polymer and consequently the extrusion temperature can be reduced to temperatures lower than 70°C; by using ethanol at concentrations higher than between 3 and 4% by weight, this temperature can be reduced to values lower than 60°C.

The Applicant has also found that using ethanol at concentrations between 5 and 10% on the weight of plasticized polymer, the extrusion temperature can be reduced to 40°C (i.e. a temperature compatible with most thermolabile biological active principles).

The plasticized polymer according to the present invention therefore contains ethanol in concentrations preferably between 5 and 10%, when used for preparing compositions for subcutaneous implants containing thermolabile active principles. Preferably in stage (b) the amount of ethanol added is equal to 10 parts by weight per parts by weight of PLGA.

In stage (c) drying is conducted until obtaining a concentration of ethanol in PLGA preferably comprised between 10 and 30%, more preferably 20% by weight /PLGA weight. Preferably drying in step (c) is carried out under an air stream at a temperature comprised between 20 and 25°C.

The temperature of grinding in stage (d), (e) and (g) is preferably -10°C, while in stage (e) the weight ratio of PLGA originating from stage (d)/PLGA as such is preferably between 16:84 and 40:60.

By increasing in stage (e) the concentration by weight of PLGA treated with ethanol with respect to the untreated PLGA, the extrusion temperature of the subsequent subcutaneous implant preparation phase is reduced.

The subcutaneous implants, a further aspect of the present invention, are prepared by a process comprising the following stages:

i) mixing the active principle with the plasticized PLGA of the present invention, at a temperature between -20°C and +5°C.

ii) extruding the ground product originating from stage (i) at a temperature less than 70°C, preferably less than 60°C.

5 As stated above, when the plasticized polymer used in stage (i) contains between 5 and 10% of ethanol, the extrusion temperature in stage (ii) is about 40°C.

In this case the aforesaid process is particularly suitable for preparing subcutaneous implants comprising thermolabile active principles. The term "thermolabile active principles" means active principles which must be stored at  
10 low temperature and in particular proteins (hormones, growth factors, enzymes etc), vaccines, antibodies and vectors for genic therapy.

The polymer plasticized with ethanol according to the present invention can also be used for preparing subcutaneous implants containing non-thermolabile active principles, however in any event as a precaution it is preferable not to subject  
15 them to sudden temperature changes.

Some illustrative but non-limitative examples of the preparation of plasticized polymer containing ethanol of the present invention together with the preparation of the subcutaneous implant of the present invention containing PLGA plasticized with ethanol and containing a thermolabile active principle are reported herein  
20 below.

EXAMPLE 1 – Preparation of subcutaneous implants containing recombinant human Granulocyte Colony Stimulating Factor (r-Hu-G-CSF)

a) Preparation of PLGA plasticized with ethanol

PLGA having the following characteristics:

25 inherent viscosity 0.19 dl/g measured at 25°C in chloroform (c = 0.1 g/dl),  
Lactide / Glycolide Molar ratio: 53 / 47,  
Tg: 40°C.

The ground product is added to an excess of ethanol until a concentration of PLGA in ethanol equal to 0.12 g/ml is obtained and is placed in a bath of water  
30 heated to 45°C and stirred for 1 minute. The ethanol diffuses into the polymer and forms a viscous gel. This gel is maintained in ethanol for 3 minutes.

This is followed by drying at 20°C until a PLGA is obtained containing ethanol in a

quantity equal to 20% weight/weight.

The polymer thus obtained is mixed at -10°C with the same untreated type of polymer as such in a weight ratio of 40:60 and the said mixture is then extruded at 75°C.

- 5 The extruded product is then ground at -10°C to obtain the plasticized PLGA with an ethanol content of 8% mass/mass.

b) Preparation of the subcutaneous implant

The active principle consisting of the protein r-Hu-G-CSF having the following characteristics:

- 10 Protein content (Colorimetry – Bradford): 2.1 to 2.6 % mass/mass,  
Biological potency (In vitro – Std WHO # 88/502): 21 to 31 x 10<sup>6</sup> IU / mg of protein,

Excipients:

- 15 Mannitol / Polysorbate 80 / Sodium Phosphate monobasic / Sodium Phosphate Dibasic Dodecahydrated / Human Albumin  
(93.4% / 0.01% / 1.9 % / 0.5% / 1.9% mass/mass respectively)

and the plasticized polymer (PLGA) were mixed intimately at -10°C in a weight ratio of 31:69 respectively.

- 20 The powdered mixture thus obtained was extruded at 40°C. The extruded product thus obtained (1.5 mm in diameter) was then cut to a length of 18 mm to form cylindrical deposits each weighing 40 mg and each containing the protein in a quantity equal to 6.6 x 10<sup>6</sup> IU.

EXAMPLE 2 – Preparation of subcutaneous implants containing recombinant human Granulocyte Colony Stimulating Factor (r-Hu-G-CSF)

- 25 a) Preparation of PLGA plasticized with ethanol

PLGA having the same characteristics as the one described in the Example 1 is ground until a particle size of less than 250 µm is obtained.

- 30 The ground product is added to an excess of ethanol until a concentration of PLGA in ethanol equal to 0.12 g/ml is obtained and is placed in a bath of water heated to 45°C and stirred for 1 minute. The ethanol diffuses into the polymer and forms a viscous gel. This gel is maintained in ethanol for 3 minutes.

This is followed by drying at 20°C until a PLGA is obtained containing ethanol in a

quantity equal to 20% weight/weight.

The polymer thus obtained is mixed at -10°C with the same untreated type of polymer as such in a weight ratio of 32.5:67.5 and the said mixture is then extruded at 75°C.

- 5 The extruded product is then ground at -10°C to obtain the plasticized PLGA with an ethanol content of 6.5% mass/mass.

b) Preparation of the subcutaneous implant

- The active principle consisting of the protein r-Hu-G-CSF having the same characteristics that the one described in the Example 1 and the plasticized polymer (PLGA) were mixed intimately at -10°C in a weight ratio of 30:70  
10 respectively.

- The powdered mixture thus obtained was extruded at 50°C. The extruded product thus obtained (1.5 mm in diameter) was then cut to a length of 18 mm to form cylindrical deposits each weighing 40 mg and each containing the protein in a  
15 quantity equal to  $6.6 \times 10^6$  IU.

EXAMPLE 3 – Preparation of subcutaneous implants containing recombinant human Granulocyte Colony Stimulating Factor (r-Hu-G-CSF)

a) Preparation of PLGA plasticized with ethanol

- PLGA having the same characteristics as the one described in the Example 1 is  
20 ground until a particle size of less than 250 µm is obtained.

The ground product is added to an excess of ethanol until a concentration of PLGA in ethanol equal to 0.12 g/ml is obtained and is placed in a bath of water heated to 45°C and stirred for 1 minute. The ethanol diffuses into the polymer and forms a viscous gel. This gel is maintained in ethanol for 3 minutes.

- 25 This is followed by drying at 20°C until a PLGA is obtained containing ethanol in a quantity equal to 20% mass/mass.

The polymer thus obtained is mixed at -10°C with the same untreated type of polymer as such in a weight ratio of 16:84 and the said mixture is then extruded at 75°C.

- 30 The extruded product is then ground at -10°C to obtain the plasticized PLGA with an ethanol content of 3.2% mass/mass.

b) Preparation of the subcutaneous implant

The active principle consisting of the protein r-Hu-G-CSF having the same characteristics as the one described in the Example 1 and the plasticized polymer (PLGA) were mixed intimately at -10°C in a weight ratio of 30:70 respectively.

The powdered mixture thus obtained was extruded at 60°C. The extruded product  
5 thus obtained (1.5 mm in diameter) was then cut to a length of 18 mm to form cylindrical deposits each weighing 40 mg and each containing the protein in a quantity equal to  $6.6 \times 10^6$  IU.

**EXAMPLE 4 – Preparation of subcutaneous implants containing recombinant human Growth Hormone (r-Hu-GH)**

10 **a) Preparation of PLGA plasticized with ethanol**

PLGA having the following characteristics is ground until a particle size of less than 250 µm is obtained.

inherent viscosity 0.19 dl/g measured at 25°C in chloroform (c = 0.1 g/dl),

Lactide / Glycolide Molar ratio: 53 / 47,

15 Tg: 40°C.

The ground product is added to an excess of ethanol until a concentration of PLGA in ethanol equal to 0.12 g/ml is obtained and is placed in a bath of water heated to 45°C and stirred for 1 minute. The ethanol diffuses into the polymer and forms a viscous gel. This gel is maintained in ethanol for 3 minutes.

20 This is followed by drying at 20°C until a PLGA is obtained containing ethanol in a quantity equal to 20% mass/mass.

The polymer thus obtained is mixed at -10°C with the same untreated type of polymer as such in a weight ratio of 40:60, and the said mixture is then extruded at 75°C.

25 The extruded product is then ground at -10°C to obtain the plasticized PLGA with an ethanol content of 8% mass/mass.

**b) Preparation of the subcutaneous implant**

The active principle consisting of the protein r-Hu-GH having the following characteristics:

30 Related proteins (Liquid Chromatography according to the monograph "Somatropin" - Nr 0951 of the 4th Edition of the European Pharmacopoeia): maximum 13% (peaks area),

Dimer and related substances of higher molecular mass (Size Exclusion Chromatography according to the monograph "Somatropin" - Nr 0951 of the 4th Edition of the European Pharmacopoeia): maximum 6% (peaks area),

Protein Content (Size Exclusion Chromatography according to the  
5 monograph "Somatropin" - Nr 0951 of the 4th Edition of the European Pharmacopoeia): 4.5 to 5.3% mass/mass,

Biological potency (Size Exclusion Chromatography according to the monograph "Somatropin" - Nr 0951 of the 4th Edition of the European Pharmacopoeia): 2.7 to 3.2 IU / mg of protein,

10 Excipients:

Glycin / Sodium Phosphate monobasic / Sodium Phosphate Dibasic Dodecahydrated

(91.4% / 1.0% / 2.5% mass/mass respectively)

and the plasticized polymer (PLGA) were mixed intimately at -10°C in a weight  
15 ratio of 30:70 respectively.

The powdered mixture thus obtained was extruded at 40°C. The extruded product thus obtained (1.5 mm in diameter) was then cut to a length of 18 mm to form cylindrical deposits each weighing 40 mg and each containing the protein in a quantity equal to 1.8 IU.

20 The integrity of the protein within the depots was examined through the following analytical package:

- Related proteins by Liquid Chromatography according to the monograph "Somatropin" (Nr 0951) of the 4th Edition of the European Pharmacopoeia,
- Dimer and related substances of higher molecular mass by Size Exclusion  
25 Chromatography according to the monograph "Somatropin" (Nr 0951) of the 4th Edition of the European Pharmacopoeia,
- Assay by Size Exclusion Chromatography according to the monograph "Somatropin" (Nr 0951) of the 4th Edition of the European Pharmacopoeia.

30 EXAMPLE 5 – Preparation of subcutaneous implants containing recombinant human Growth Hormone (r-Hu-GH)

a) Preparation of PLGA plasticized with ethanol

PLGA having the same characteristics as the one described in the Example 4 is



ground until a particle size of less than 250  $\mu\text{m}$  is obtained.

The ground product is added to an excess of ethanol until a concentration of PLGA in ethanol equal to 0.12 g/ml is obtained and is placed in a bath of water heated to 45°C and stirred for 1 minute. The ethanol diffuses into the polymer and forms a viscous gel. This gel is maintained in ethanol for 3 minutes.

This is followed by drying at 20°C until a PLGA is obtained containing ethanol in a quantity equal to 20% mass/mass.

The polymer thus obtained is mixed at -10°C with the same untreated type of polymer as such in a weight ratio of 32.5:67.5 and the said mixture is then extruded at 75°C.

The extruded product is then ground at -10°C to obtain the plasticized PLGA with an ethanol content of 6.5% mass/mass.

#### b) Preparation of the subcutaneous implant

The active principle consisting of the protein r-Hu-GH having the same characteristics as the one described in the Example 4 and the plasticized polymer (PLGA) were mixed intimately at -10°C in a weight ratio of 30:70 respectively.

The powdered mixture thus obtained was extruded at 50°C. The extruded product thus obtained (1.5 mm in diameter) was then cut to a length of 18 mm to form cylindrical deposits each weighing 40 mg and each containing the protein in a quantity equal to 1.8 IU.

The integrity of the protein within the depots was examined through the same analytical package that the one described for Example 4.

### EXAMPLE 6 – Preparation of subcutaneous implants containing recombinant human Growth Hormone (r-Hu-GH)

#### a) Preparation of PLGA plasticized with ethanol

PLGA having the same characteristics as the one described in the Example 4 is ground until a particle size of less than 250  $\mu\text{m}$  is obtained.

The ground product is added to an excess of ethanol until a concentration of PLGA in ethanol equal to 0.12 g/ml is obtained and is placed in a bath of water heated to 45°C and stirred for 1 minute. The ethanol diffuses into the polymer and forms a viscous gel. This gel is maintained in ethanol for 3 minutes.

This is followed by drying at 20°C until a PLGA is obtained containing ethanol in a quantity equal to 20% mass/mass.

The polymer thus obtained is mixed at -10°C with the same untreated type of polymer as such in a weight ratio of 16:84 and the said mixture is then extruded at  
5 75°C.

The extruded product is then ground at -10°C to obtain the plasticized PLGA with an ethanol content of 3.2% mass/mass.

**b) Preparation of the subcutaneous implant**

The active principle consisting of the protein r-Hu-G-CSF having the same  
10 characteristics as the one described in the Example 4 and the plasticized polymer (PLGA) were mixed intimately at -10°C in a weight ratio of 30:70 respectively.

The powdered mixture thus obtained was extruded at 60°C. The extruded product thus obtained (1.5 mm in diameter) was then cut to a length of 18 mm to form cylindrical deposits each weighing 40 mg and each containing the protein in a  
15 quantity equal to 1.8 IU.

**EXAMPLE 7 – Preparation of subcutaneous implants containing Interferon alfa-2a**

**a) Preparation of PLGA plasticized with ethanol**

PLGA having the following characteristics is ground until a particle size of less  
20 than 250 µm is obtained.

inherent viscosity 0.19 dl/g measured at 25°C in chloroform (c = 0.1 g/dl),  
Lactide / Glycolide Molar ratio: 53 / 47,  
Tg: 40°C.

The ground product is added to an excess of ethanol until a concentration of  
25 PLGA in ethanol equal to 0.12 g/ml is obtained and is placed in a bath of water heated to 45°C and stirred for 1 minute. The ethanol diffuses into the polymer and forms a viscous gel. This gel is maintained in ethanol for 3 minutes.

This is followed by drying at 20°C until a PLGA is obtained containing ethanol in a quantity equal to 20% mass/mass.

30 The polymer thus obtained is mixed at -10°C with the same untreated type of polymer as such in a weight ratio of 40:60 and the said mixture is then extruded at 75°C.

The extruded product is then ground at -10°C to obtain the plasticized PLGA with an ethanol content of 8% mass/mass.

**b) Preparation of the subcutaneous implant**

The active principle consisting of the protein Interferon alfa-2a having the following characteristics:

Related proteins (Liquid Chromatography according to the monograph "Interferon alfa-2 Concentrated solution" - Nr 1110 of the 4th Edition of the European Pharmacopoeia): maximum 5% (peaks area),

Protein Content: 1.8% mass/mass,

Biological potency (Size Exclusion Chromatography according to the monograph "Interferon alfa-2 Concentrated solution" - Nr 1110 of the 4th Edition of the European Pharmacopoeia):  $2.3 \times 10^8$  IU / mg of protein,

Excipients:

Sodium Acetate / Sodium Chloride / Trehalose

(5.9% / 8.4% / 83.9% mass/mass respectively)

and the plasticized polymer (PLGA) were mixed intimately at -10°C in a weight ratio of 25:75 respectively.

The powdered mixture thus obtained was extruded at 40°C. The extruded product thus obtained (1.5 mm in diameter) was then cut to a length of 18 mm to form cylindrical deposits each weighing 40 mg and each containing the protein in a quantity equal to  $40 \times 10^6$  IU.

The integrity of the protein within the depots was examined through the following analytical package:

- Related proteins by Liquid Chromatography according to the monograph "Interferon alfa-2 Concentrated solution" (Nr 1110) of the 4th Edition of the European Pharmacopoeia.